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THE GENERAL FORM OF EXCRETION IN THE LOBSTER, HOMARUS [1]

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While there exists an extensive literature on the regulation of inorganic ions in the higher Crustacea (Krogh, 1939; Robertson, 1949, 1953; Prosser *et al.,* 1950), the experimental study of nephridial function has received little attention, due apparently to a lack of a method for securing repeated samples of urine. This report gives such a method and it is an attempt to develop an integrated picture of the regulation of the internal environment of a single species, *Homarus americanus* (a lobster), through the experimental study of a variety of organic and inorganic substances.

Comprehensive inorganic analyses of lobster blood have been made by Cole, and Smith in Cole (1940), and of blood and urine by Robertson (1939, 1949, 1953), and by Robertson and Webb (1939). The nephridial anatomy has been described by Marchal (1892), Waite (1899), and Peters (1935). Histologically there is no structure corresponding to the vertebrate glomerulus. The study by Cuénot (1895) of the differential concentration of dyes by various organs in different decapods is of considerable interest.

Materials and Methods

Because of the large number of substances studied and of experiments performed, individual techniques will be described in context. Over two hundred lobsters were studied. These were largely from the Mt. Desert Island region, but a few were from Nova Scotia. For all experiments the animals were given at least twenty-four hours to equilibrate to the sea water at the Laboratory.

Urine from the nephridial bladders was secured at will without catheters by a technique shown to us by Dr. P. R. Wilder of the Atlantic Biological Laboratory, St. Andrews, N. B. If one's thumb is placed between the bases of the pereiopods with the fingers over the carapace, preferably with the tail flexed, squeezing the hand results in two jets of urine from the nephridiopores which can be collected in test tubes held by the other hand. This technique works best with so-called "hard-shelled" lobsters. The value of the technique was tested critically with twelve lobsters. After expressing the urine, the carapace was cut open and the bladders were examined directly. In eight animals the bladders were empty; the highest residuum

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of the remaining four was 10% of the expressed volume. Thus the technique, while not perfect, is serviceable. Since not all lobsters from commercial pounds form urine, and since individual animals differ in the ease with which urine can be expressed, another critical study of twelve lobsters was made which showed we could differentiate accurately the anuric specimens. As with many other techniques, judgments depend more on practice than on formal rules. In general, however, if the animal has good muscle tone, if the opercular flaps of the nephridiopores are elevated on squeezing, and if the lobster is fresh from sea water, the animal is producing urine. It is necessary sometimes to flick the opercular flaps with the fingernail to start the flow of urine.

Safe occlusion of the nephridiopores can be made by placing a wide rubber band across the pores, crossing the band over the dorsal carapace, and then securing the band on the posterio-ventral margin of the gill covers.

Test substances can be injected into the hemocoele, or more safely can be pipetted through the mouth into the stomach from which they are absorbed. Blood is most easily withdrawn from the ventral surface of the abdomen. The only reliable method we found for the prevention of clotting was to whip or shake the blood, filter it several times (Cole and Kazalski, 1939), and then preferably dilute it.

Results

The data [2] are presented in three sections: Organic Substances, Inorganic Substances, Intake and Output, with the discussion pertinent to each *in situ*.

Organic substances

1. *Inulin.* Single injections into eight lobsters of inulin were followed up to 28 hours with up to 5 sampling periods. The analytic method of Schreiner (1950) was used. For blood levels which ranged between 17 and 1.4 mg. %, the urine-plasma (U/P) ratios were essentially one (1.0–1.1), with the concentrations in the blood and urine falling with the same slope. Forster and Zia-Wohlrath (1941), working with higher blood inulin levels (192–65 mg. %), also found inulin U/P ratios of one. In the lobster, inulin is not secreted by the nephridium as reported for the crayfish (Maluf, 1941). Since the inulin U/P ratio is substantially one, the U/P ratios alone of other test substances should offer a reasonably accurate guide for determining the partitioning ability of the nephridium, and there is no need to compare the concentration of a test substance with the urinary concentration of inulin in subsequent experiments, as would be necessary if there were a differential separation of water and inulin.

2. *Vertebrate hemoglobin and plasma proteins.* To test the permeability of the nephridium to large molecules, solutions of hemoglobin prepared from hemolyzed red cells of the dogfish (*Squalus acanthias*) were injected into the hemocoele of eight lobsters. The normally clear urine promptly became pink and remained so for several days. Plasma proteins from the dogfish were injected into four lobsters. On gentle evaporation, the urine jelled, an unnatural event. While it was not de-

[2] The following technical assistance is acknowledged gratefully: Dr. E. L. Becker, freezing point depression; Dr. Klaus Brunn and Xenia Boysen, urea; Dr. Roy P. Forster, inulin and PAH; Drs. Henry Heinemann and Wilbur Sawyer, chloride; Drs. Martin Rubin and Frederick Berglund, magnesium and calcium; Dr. Charles G. Zubrod, glucose.

termined that the original molecules were recovered in the urine in their original state, it does appear that the nephridium of the lobster is permeable to molecules of the size found in the blood of fish. The natural urine of the lobster is protein-free (Forster and Zia-Wohlrath, 1941). Urine treated with the standard protein-precipitating agents shows no increased Tyndall effect or clouding.

3. *Glucose.* The Hagedorn-Jensen sugar titration method (Peters and Van Slyke, 1946) gave in mg. % urine/blood values of: 0/22; 0/24; 0/27; 0/28; 0/32; 0/37; 0/39; 0/40. Two urine values were slightly positive: 2/31; < 5/31. It is

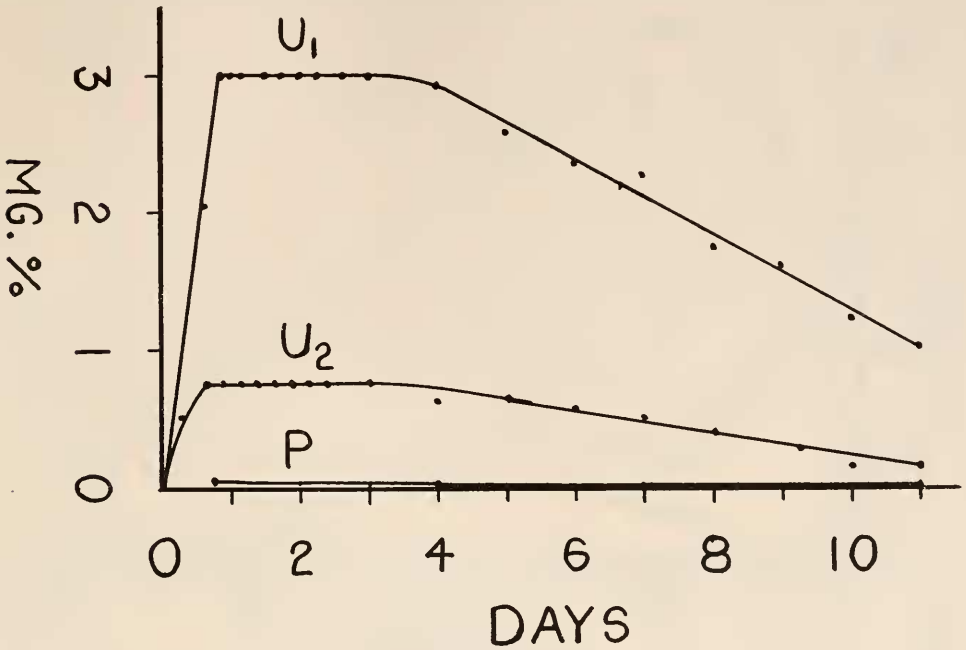

FIGURE 1. Concentrations of phenol red in urine (U_1, U_2) and blood (P) in two lobsters with 6 and 4 mg. of dye pipetted into the stomach. The blood levels were so similar that they are given as one curve, scarcely distinguishable from the horizontal axis. At these blood levels the dye is obviously secreted by the nephridia, but leaves the animal slowly for reasons explained in the text.

uncertain whether these are genuinely positive values since the method involves subtracting from the titrated value the value of a blank. Morgulis (1922) reported previously blood "glucose" levels of 19–26 mg. % for *Homarus,* with a considerable variability in other decapods (1922, 1923). That the test was measuring sugar and not some other reducing substance is indicated by the fact that in lobsters with the heart destroyed, the blood was free from reducing substance fifteen minutes later.

With the injection of exogenous glucose, the lobster's nephridium behaves like the vertebrate kidney. Up to blood levels of about 100 mg. % the urine remains free of glucose. Urine-plasma ratios were: 0/81; 0/84; 0/94; 0/106. With further elevation, glucose spilled into the urine, *e.g.,* 30/202; 30/210, and at blood concentrations of 400–500 mg. % the U/P ratio approached one. The report by For-

ster and Zia-Wohlrath (1941) of a glucose U/P ratio of one in *Homarus* is due undoubtedly to the high level of glucose employed.

Phlorizin resulted in glycosuria with or without priming by sub-liminal exogenous glucose (10 lobsters). In short, under normal conditions there seems to be an active mechanism which excludes glucose from the urine, a mechanism which can be poisoned by phlorizin.

4. *Phenol red.* Phenol red was extracted from the blood by acid alcohol, read colorimetrically at 440 mμ, alkalized and read again at 550 mμ. Urine and stomach fluid were treated similarly. It was found desirable to use control blanks from in-

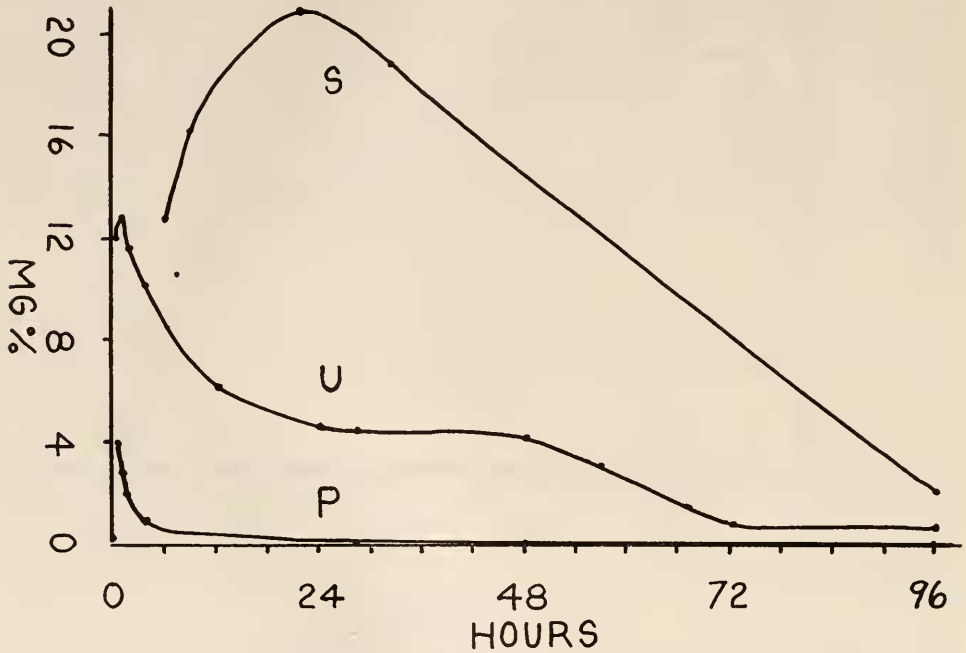

FIGURE 2. Urinary concentrations (U) of phenol red after injection into the hemocoele, over wider blood levels (P) than shown in Figure 1. Note the concentration of the dye in the digestive fluid (S). The isolated point is a urine value.

dividual lobsters, and not to rely on a generalized control zero. Over thirty animals were studied.

The pattern of nephridial excretion of phenol red is seen in Figures 1 and 2. At low plasma concentrations (Figs. 1, 2), the dye is clearly concentrated by the nephridium (the U/P ratio is a valid criterion; see paragraph on inulin). As the plasma level is raised, the U/P ratio approaches one (*cf.* Fig. 4). This is the pattern for all substances "secreted" by the nephridium; concentration is evident at low plasma concentrations but is apparently swamped by high plasma concentrations. It is to be remembered that the lobster's kidney is the elaboration of only one pair of nephrons.

Phenol red is not lost or absorbed through the gills or body covering. Four lobsters were injected with 5–10 mg. and were placed for 18 hours in volumes of

water sufficiently small to detect a small fraction of the injected dye. No phenol red was detected in the external medium. Four animals were placed for 18 hours in sea water containing 300 mg. % phenol red. No dye was detectable in the blood, urine, or digestive gland. Phenol red placed in the stomach is absorbed. After injection of phenol red, this substance was detectable in the cells of the gills. The uptake of dye by branchial cells is seen more dramatically with Evans blue. After a single injection of Evans blue, the gills become a bright blue, and so remain for at least a month, when no dye is detectable in the blood. The dye obviously entered

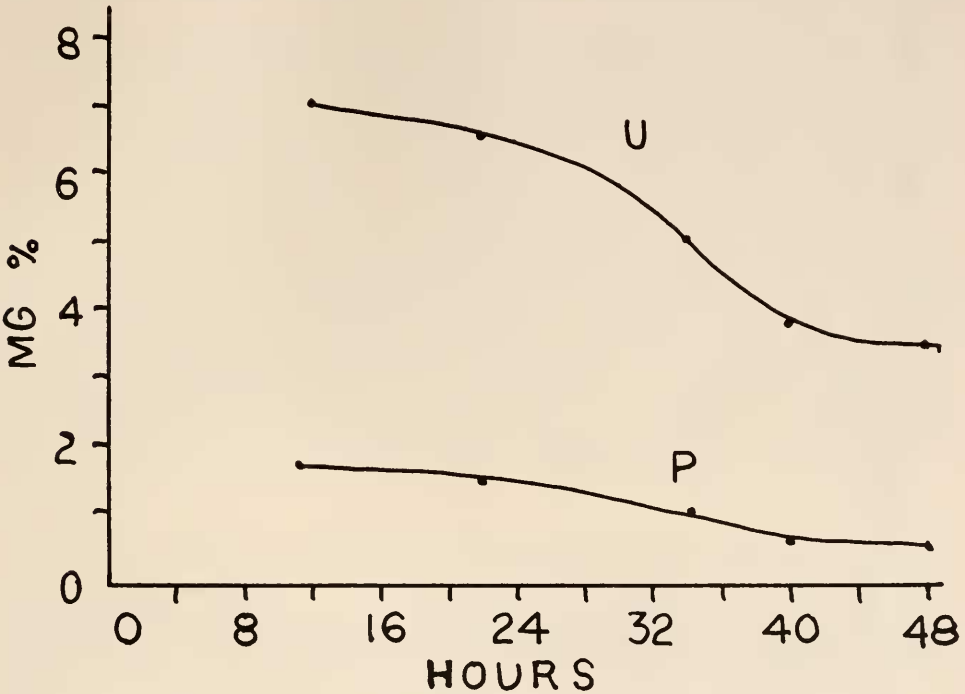

FIGURE 3. Concentrations of para-aminohippurate in urine (U) and blood (P) after a 4-mg. stomach infusion. Nephridial secretion is evident.

the cells of the gills, but did not move easily either out to the sea water or back to the blood. One can speculate that for certain substances passage through the branchial cells involves at least two steps: cellular uptake or penetration, and extrusion.

Fecal loss of phenol red is minute. The dye, however, is taken up by the digestive gland. Extracts from the gland show phenol red when after injection the blood level has subsided to insignificant amounts. The dye absorbed by the digestive gland is secreted with the digestive juice at concentrations greater than those of the urine (Fig. 2). Whether the individual cell of the digestive gland can concentrate more than the nephridial cell requires further study, since the mass of the digestive gland is greater than that of the kidney, and the movement of water through these two types of cells probably is not the same.

The dye secreted with the digestive juice is not lost with the feces or voided by

mouth, but is reabsorbed, and cycles back and forth between the stomach and the digestive gland, *slowly* being lost with the urine (Fig. 1). The nephridia seem, therefore, the principal port of exit for phenol red.

5. *Sulfobromophthalein* (*bromsulfalein*). This dye, analyzed colorimetrically, was explored over blood concentration of 0.02–100 mg. % in eight lobsters. The U/P ratios were between one and two with no tendency to rise at the lower plasma concentrations. It is concluded that bromsulfalein is not concentrated by the nephridium. In the digestive juice, however, bromsulfalein is concentrated more

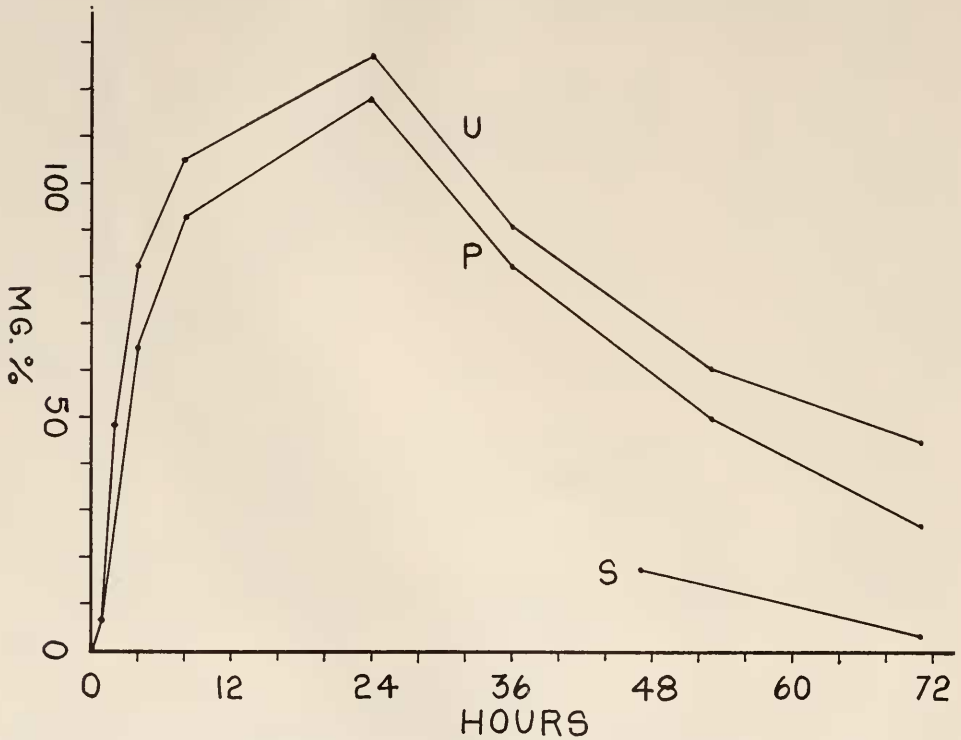

FIGURE 4. Urine (U) blood (P), and digestive juice (S) values following a 400-mg. dose of PAH, placed in the stomach. At high blood levels the U/P ratio approaches one. As the blood level falls, the U/P ratio increases. This sort of curve is characteristic for substances secreted by the nephridia.

strongly than phenol red. Without further quantitation, a numerical comparison is dangerous, but with comparable doses (10, 25 mg.) of dyes into lobsters of comparable weight, the digestive juice bearing bromsulfalein showed about four times the concentration of dye as that bearing phenol red. The differential ability of the hepato-pancreas to concentrate this dye like the vertebrate liver indicates that the name for this organ is more than an anatomical appellation. Like phenol red, the loss from the lobster of bromsulfalein is very slow.

6. *Para-aminohippurate* (PAH). Using the analytic method of Smith *et al.* (1945), nephridial concentrations were explored in eight lobsters with blood levels

of 120–0.5 mg. %. At low blood levels (Fig. 3), PAH is concentrated in the urine and the U/P ratio rises as the blood level falls. As the blood concentration is raised the U/P ratio approaches one (Fig. 4). The digestive gland does not concentrate PAH; rather, the stomach juice concentration is below the blood concentration.

7. Urea and nitrogen. Analyses for urea and volatile ammonia were made by the method of Seligson and Seligson (1951). For non-protein nitrogen (NPN) sulphuric acid digests were nesslerized directly. Urea and volatile ammonia were undetectable in blood and urine (10 animals). The natural range for NPN was 5–32 mg. %. Morgulis (1922) found a range of 12.5–13.3 mg. % for *Homarus,* with a wider range in other decapods. No constant relationship was observed between blood and urine NPN. The U/P ratios varied from 0.7–6.0 in ten animals.

Exogenous urea is lost rapidly from the blood through the gills. Four lobsters with occluded nephridiopores were injected with 500 mg. urea, then placed in measured volumes of sea water. After one hour sea water/blood concentrations in mg. % fell between 12.8/12.65 and 19.6/14.25. Since the external volume was greater than that of the animals, most of the urea was in the external medium. In four other animals injected with 500 mg. urea and with occluded nephridiopores, no urea was detectable in the blood after the animals were 24 hours in running sea water. Morgulis (1922) found a rapid disappearance of blood urea and ammonium sulphate without nephridial occlusion in *Panulirus.* While currently there is no evidence that urea is the main nitrogen excretory product, the above experiments indicate a high in-out permeability to urea by the gills, and that this loss can occur without nephridial participation. The presence of a substantial blood NPN must indicate a low branchial permeability to certain nitrogenous compounds.

Inorganic substances

Chemical analyses for sea water, blood, and urine are given in Table I. In the paragraphs following are data on individual ions. The purpose of this study is to understand the range of capacities for dealing with various ions and their ports of entrance and exit. Robertson's studies (1939, 1949, 1953; Robertson and Webb, 1939) have defined at a non-experimental level the natural partition ratios for *Homarus vulgaris.* Robertson (1949) has criticized correctly the presentation of blood values in millimols/liter, and has emphasized the need for a correction for the presence of blood proteins. In working up his ratios he seems to have used a single average value of 29 g./liter protein for *Homarus,* although for a variety of decapods he found values ranging from 29–80 g./liter. Allison and Cole (1940) got values of 17.1–31.2 g./liter of hemocyanin for Mt. Desert Island lobsters. Our data from over thirty Mt. Desert Island lobsters show a very high variability in what we are somewhat arbitrarily calling blood protein. Values ranged from 11–62 g./liter, with about 50 g./liter as a generalized usual figure. The above values were secured, following one of the methods of Robertson (1949), by drying weighed calibrated volumes of serum to constant weight at 100° C. From the residual weight was subtracted the weight of electrolytes (Na, K, Ca, Mg, Cl, PO_4, SO_4), an arbitrary value for the water of crystallization derived from dried sea water, known organic substances such as NPN and glucose. In the absence of any evidence in the literature of massive amounts of some unknown substance, and since

known organic substances occur in fractions of a gram/liter, the values here presented while perhaps not entirely pure, do seem to offer a fair picture of the order of magnitude and of the natural range of blood proteins. It perhaps should be emphasized the values presented represented a selection of living lobsters some of which were lethargic and sub-standard. The range for so-called normal lobsters was 35–62 g./liter.

It is obvious that unless the non-electrolyte concentration of the blood is determined for each *animal,* molal expressions for electrolytes are not entirely precise.

TABLE I

Analyses of lobster blood, urine, and of sea water

	Sea water		Blood		Urine	
pH*	(7.6–8.0)	[10]	(7.45–7.6)	[16]	(7.4–7.55)	[26]
Dried solids,** g./liter	(35.5–35.9)	[10]	(71.2–98.6→47)	[22]	(32.7–35.9)	[8]
Organic solids, g./liter	—		50 (35–62→11)	[22]	(<0.4)	[8]
Water, g./liter of blood	—		950 (935–959→976)	[22]	—	
Milliosmols	(915–920)	[12]	(920–952)	[20]	(918–950)	[20]
Sodium, mM/liter	440 (428–445)	[15]	472 (451–488)	[20]	474 (454–486)	[22]
Potassium, mM/liter	(9–10)	[10]	(6–11)	[14]	(4–10)	[16]
Magnesium, mM/liter	(50–52)	[8]	6.8 (5.4–8.6)	[15]	11.4 (7.2–17.6)	[15]
Calcium, mM/liter	(9–10)	[8]	15.6 (13.1–18.6)	[15]	12.7 (5.6–16.3)	[15]
Chloride, mM/liter	503 (476–515)	[27]	470 (465–490)	[27]	505 (490–520)	[27]
Phosphate, g./liter	—		0.016 (0.008–0.018)	[8]	0	[16]
"Glucose," g./liter	—		(0.22–0.40)	[10]	(0–0?)	[10]
Non-protein nitrogen, g./liter	—		(0.05–0.32)	[10]	(0.05–0.32)	[10]
Volatile ammonia	0	[4]	0	[10]	0	[12]
Urea	0	[4]	0	[10]	0	[12]

Figures within parentheses give the range of values. These fluids are to be considered as having a natural variability and are not of constant composition. The above ranges exceed the experienced variability of the methods. Figures outside of parentheses are average values. Figures to the right of arrows are unusual values. Figures in brackets show the number of samples (sea water) or the number of animals analyzed. The various zeros are zeros for the method used. Analyses were done in duplicate or triplicate.

* Measured by Beckman and Cambridge meters. Sea water from the Bay was about 8.0; the running laboratory water was variable.

** Includes water of crystallization.

Some of these data were secured over several years, some in a single summer season, and some in shorter periods. For this reason they should be viewed only as general parameters. For critical quantitative work complete work-ups of individual animals should be made.

For this reason we have presented our data in Table I in the raw form of mM/liter. A crude generalized correction can be made by taking the blood protein as 50 g./liter.

1. *Phosphate.* Fiske-SubbaRow phosphate (method given by Hawk *et al.,* 1947) was absent from sixteen urines. Blood levels of eight lobsters were about 1.6 mg. % (range: 0.8–1.8). If exogenous inorganic phosphate (sodium salts mixed to a slightly alkaline pH) is injected, phosphate spills over into the urine. With blood levels of about 8 mg. %, phosphaturia occurred. The nephridium behaves toward inorganic phosphate as it does to glucose.

2. *Magnesium and calcium.* An ethylenediamine tetraacetic acid (EDTA) titration, developed by Dr. Martin Rubin (personal communication), was used. Calibration studies indicated that the method is serviceable if one discards all samples which do not titrate sharply. The method gives initially combined magnesium and calcium, then magnesium. Calcium is obtained by subtraction.

The analyses of Cole, Smith in Cole (1940) showed there is a marked partitioning between sea water and blood in *Homarus*. Robertson (1953) found a molal magnesium U/P ratio of 1.8, and a calcium U/P ratio of 0.64. Our data on fifteen lobsters give the following molar ratios (see above): blood/sea water: Mg + Ca, 0.37; Mg, 0.13; Ca, 1.68. Urine-plasma ratios were: Mg + Ca, 1.16 (range: 1–1.3); Mg, 1.7 (range: 1–2.6); Ca, 0.81 (range: 0.53–1). In most animals magnesium is concentrated in the urine and calcium is reduced. There are instances, however, where each ion is not affected (U/P ratio of one), and there was one animal where both the magnesium and calcium U/P ratio was one. In dilute sea water, the magnesium U/P ratio falls, and may drop to 0.8. In short, some qualification must be placed on the idea that the lobster's nephridium concentrates magnesium in the urine, and conserves calcium, although this is the usual situation.

The gills and carapace are relatively impermeable to magnesium. There was no elevation in blood or urinary magnesium in four lobsters placed for twelve hours in baths made of half sea water with magnesium chloride added to return the water to near its original equivalence. In lobsters naturally anuric, blood magnesium is at normal levels. There seems no tendency in these animals for magnesium to build up in the blood as might be expected if the gills were permeable inwardly to magnesium.

Since the lobster usually produces urine, it is losing magnesium with the urine. The daily urinary loss approximately equals the magnesium found in 5 ml. of sea water. The port of entrance seems to be the stomach. With urinating lobsters sea water is drunk intermittently with the food or on an empty stomach (see section on Intake and Output), and this sea water is absorbed. The stomach normally contains a concentration of magnesium greater than that of the blood, although in unfed lobsters empty stomachs are found frequently. Sea water placed in the stomach is slowly absorbed, and as shown below, the stomach fluid does not furnish the bulk of the fluid for the urine (24 ml./*diem*). It must not be thought that there is a steady rapid movement of magnesium-laden fluid into the blood from the stomach which would result in high levels of blood magnesium. The anuric animals mentioned above had empty stomachs.

With urinating lobsters, injected magnesium sulphate or chloride results in an increased magnesium excretion although the U/P ratios do not rise above those found normally; magnesium sulphate has a marked diuretic and then an anesthetic effect. Exogenous magnesium placed in the stomach is absorbed (see Intake and Output). In dilute sea water lobsters conserve magnesium. Four animals kept for 24 or 48 hours in 60–70% sea water had urinary levels lower than plasma levels. Blood levels remained within 1 mM/liter of the levels found with full sea water. Under these same conditions blood calcium fell more obviously, 2–4 mM, and the nephridium did not conserve calcium more than it did in full sea water.

It is obvious from Table I and from the above data that the nephridium has very modest powers in partitioning these two ions (Mg, Ca). Magnesium is lost more through the volume of urine flow than by nephridial concentration. Calcium is only

weakly conserved. It should be noted that all the lobsters used were hard-shelled animals near the end of an inter-molt period.

Levels of blood magnesium can be changed without nephridial participation, at least on a short term basis. Blood levels were raised up to 33 mM/liter by placing magnesium chloride solutions in the stomach of anuric animals. Over a subsequent 11-hour period, blood magnesium fell to 10–21 mM/liter without urine production or without a rise in stomach magnesium. During this experiment, blood calcium levels remained unchanged. Either the magnesium was taken up by the tissues and carapace or it passed out through the gills.

3. *Sulphate.* Analyses were semi-quantitative. Known volumes of fluid were treated with known volumes of barium chloride in an ice bath, and the amount of precipitate was measured. The general picture for this ion is like that for magnesium. The gills and carapace are relatively impermeable to sulphate. After 18 hours, there was no elevation of blood or urinary sulphate in six lobsters placed in baths with elevated sulphate (Na, Mg). Blood and urine sulphate was elevated when sulphates were placed in the stomach. In 18 normal lobsters, the sulphate U/P ratio varied between one and two, but rose to four with small amounts of injected sulphate. With increased dosage up to blood levels of 300 mg. %, the U/P ratio fell toward one. Increased sulphate was markedly diuretic to urinating lobsters. Following the elevation of blood levels, the return to normal was slower than for magnesium; as with phenol red, days were required to effect normal blood levels. This seems to indicate that sulphate is lost primarily through the nephridia.

4. *Monovalent ions.* Natural values are given in Table I. Analyses for sodium and potassium were done by flame photometry. Chlorides were done by electrical conductivity measurements of silver nitrate titrations, or by a mercuric nitrate titration with s-diphenyl-carbazone as a visual indicator.

On the basis of a generalized calculated molality, using 50 g./liter protein, chloride ratios of sea water/blood and urine/blood are essentially one (1–1.01). This same ratio holds for serum dialyzed through Visking membranes against full strength and 60% sea water. Cole's data (1940) and Robertson (1949) give this same ratio. Under normal conditions of undiluted sea water the distribution of chloride in the blood and urine seems to be a passive one. This might be expected when one considers that the blood proteins have a negative charge and that there is no heavy concentration of a non-diffusible cation in the blood.

Cole (1941) has presented evidence that in dilute sea water the lobster can secrete chloride inwardly. Twelve lobsters whose blood/sea water molar ratio averaged 0.95 (sea water, 504 ± 9 mM/liter) were placed in dilute running sea water which did not exceed 394 mM/liter. Between 37 and 118 hours in this diluted sea water, and following an initial hemodilution, the blood/sea water ratio rose to 1.05–1.11 in five lobsters sampled.

In twenty-seven analyses of sea water, blood, and urine for normal lobsters, we found without exception that the molar blood/sea water ratio was below one, and the urine/blood ratio was above one. The millimolar differences between sea water and blood were about 25–35. Dialysis of serum of three lobsters (La, b, c) against sea water for 24 hours through Visking membranes gave the following chloride values in mM/liter: sea water, 511, 512, 518; La, 478, 478; Lb, 480, 485; Lc, 483, 487. Dialysis of serum of three lobsters (Ld, e, f) against dilute sea water gave the following values: dilute sea water, 308, 308, 311; Ld, 287, 287; Le, 288,

291; Lf, 287, 292. These values show that there is no capacity of the blood which tends to elevate blood chloride in situations where the blood is separated from full and dilute sea water by an inert membrane. Under dilute conditions, any marked rise in blood chloride can not be due to the blood itself or to an inert membrane effect.

Cole's experiment was repeated with seven lobsters (L1–7). For three lobsters (L1–3) pre-dilution values in mM/liter averaged: sea water, 510 ± 5; serum, 470 ± 5. Running dilute sea water varied between 310–338. After 72 hours, serum chloride values were: 370, 370; 357 ± 3; 355 ± 3. For L4–5, initial control values were: sea water, 500 ± 2; serum, 485 ± 5. Dilute sea water was 358–371. After 96 hours, serum values were 376, 380; 380, 384. For L6–7, control values were: sea water, 503 ± 7; serum, 485, 485; 477 ± 1. Dilute sea water was 378–392. After 116 hours in diluted sea water, serum values were: 420, 424; 412, 417. It should be noted that during the first twenty-four hours of dilution blood chloride falls below the above values, and subsequently rises.

In all the above animals in dilute sea water, the blood/sea water ratio was above one, an event which does not occur in full sea water or after dialysis with an inert membrane. It would seem that this elevation in blood chloride is due to some active process.

In all the above animals in dilute sea water, the urine chlorides were 20–33 mM/liter higher than the blood chlorides. When calculated on a molal basis, this difference disappears (see above). These data indicate that the nephridium is losing chloride at the elevated blood levels and the nephridium is not participating in the elevation of blood chloride nor is it helping to conserve chloride. In other Crustacea, nephridia are known to conserve chloride and the gills are known to secrete chloride inwardly. Here in the lobster, the nephridia clearly do not play such a role. While our data do not irrefutably establish the secretion of chloride, the most likely site for the agency effecting the elevation of blood chloride is the gills. The persistent elevated chlorides after 116 hours would seem to indicate that the higher blood chloride is not due to tissue chloride which has come out of cells but has not been carried away by the urine, diffusion, etc.

While it is clear that in full-strength sea water the distribution of chloride can be accounted for on a passive basis, the sodium picture is a bit more complex. On a molal basis there are about 60 equivalents more sodium in the plasma than in sea water. The sum of the equivalents of the major electrolyte cations of the blood (Na, K, Mg, Ca) accounted for about 99% of the cations of sea water. Since the data were worked up on an average basis the percentage figure should not be taken too literally, but merely to indicate there is not an unforeseen discrepancy between the cationic sum of blood and sea water (the blood and sea water are roughly isotonic; see below and Table I). The extra sodium of the blood can be accounted for on the basis of the exclusion of most of the magnesium of sea water from the blood. If one groups sea water magnesium and calcium, subtracts plasma magnesium and calcium and the equivalents bound to blood sulphate (arbitrarily taken as 10 mM/liter from data of Robertson and Webb, 1939 and Cole, 1940), one has about 50–60 equivalents which is the excess of sodium in the blood. Blood and sea water potassium are very close to each other (Table I).

The U/P partition is not so easily explained. There seems on a molal basis to be a persistent deficit (10–30 mEq.) of combined urinary sodium and potassium,

largely sodium. Urinary potassium tends *irregularly* to be several milliequivalents lower than plasma (not higher); combined magnesium and calcium add only several milliequivalents to urine as compared to blood. Blood/urine potassium, magnesium, and calcium thus tend to cancel each other. Robertson (1949) gives a molal U/P) ratio for sodium of 0.99, indicating again a sodium deficit. It may be that since the urine is isotonic with the blood, there is an increased activity in the urine where the cations are free from the depressing influence of the blood proteins.

The above natural data and the following experimental data indicate that sodium chloride is freely mobile across the gill-blood and blood-urine barriers. On placing lobsters in dilute sea water (60, 70, 75, 80, 90%) the osmotic uptake of water, as judged by the gain in body weight, is completed in less than one hour, with a gain of 1–2% of body weight (18 animals). While this uptake results in hemodilution, the blood sodium chloride continues to fall, approaching the concentration of the external medium in twelve hours or less. The blood concentration after the first fifteen minutes of dilution falls in an almost linear fashion with a slope varying with the amount of external dilution. Within the first three hours the bulk of the sodium chloride to be lost is lost. Blood sodium chloride begins to asymptotically approach the external concentration but remains superior to the external concentration on a molar basis. On returning the lobsters to full-strength sea water, the concentration curves are not the reverse of the dilution curves. During the first hour the blood concentration rises very steeply and asymptotically approaches normal values in three to six hours. After injecting sodium chloride sufficient to raise blood levels 15–20 mM/liter, the blood returns to normal values in three hours. Under these altered sodium chloride loads, the urine reflects the blood concentration, rising or falling in sodium chloride concentration with blood concentration or dilution. In anuric lobster, blood sodium chloride adjusts to control values following the injection of hypo- or hypertonic saline within about three hours.

The above data suggest that while under conditions of external dilution, the lobster has the capacity to actively raise its blood chloride, under normal conditions of undiluted sea water, sodium chloride is passively distributed between sea water, blood, and urine. That is, normally there is no active process which is acting on sodium chloride as such. The amount of sodium in the blood can be explained as the amount in sea water plus the equivalents replaced for the sea water magnesium which is excluded from the blood. The discrepancy between blood and urine sodium is too small to support the idea that some active partitioning of sodium is being effected by the nephridium. Indeed, the dilution experiments indicate that the nephridium does not have a chloride-conserving mechanism.

Intake and output

While it is obvious that in the natural environment, the intake of organic chemicals is by mouth, it is not recognized that in the lobster sea water enters by mouth. The stomach capacity is about 10 ml. for a 500-gram animal. On eating, the food is diluted with a good deal of sea water. But even lobsters with empty stomachs will fill the stomach with sea water. This drinking is not due to fright or experimental handling. If the stomach is emptied by pipette, some animals will immediately fill the stomach with sea water. The stomach contents are never regurgitated unless the animal is in great distress. The stomach contents are always com-

pletely absorbed. The above observations come from dozens of lobsters made over five years and are not quick impressions. Many lobsters were followed at intervals for several days. It is not to be inferred that all non-feeding lobsters keep their stomachs full of sea water. The drinking is an intermittent affair, whose cause was not determined.

Since all substances mentioned in this report, with the exception of fish protein and lobster NPN which were not studied for this purpose, were found experimentally to be absorbed from the gut when placed in the stomach, the diet and imbibed sea water contribute to the internal electrolytic and non-elecrolytic content of the lobster. Since, too, the gills and carapace were found to be relatively impermeable to magnesium and sulphate, the stomach seems to be the principal port of entrance for these substances. Possible branchial uptake of calcium and phosphate was not studied, but these obviously enter with the diet. While water and monovalent ions do enter from the stomach, this route does not appear as the principal route (see below). The principal route seems to be the gills.

The nephridia and the gills seem to form the principal points of exit for the substances under discussion. The feces were not studied critically. Their fluid volume is small and only minute amounts of phenol red or bromsulfalein are lost with the feces. There seems little likelihood that the feces play any great role with the chemicals here discussed.

The concentrating powers of the nephridium have been discussed. The daily chemical loss through the nephridium depends not only on the nephridium's partitioning powers but also on the urine flow. For so-called normal lobsters the urine output is about 1 ml./hr./0.5 kg. (determined by hourly collections over 12-hour periods), with flows up to 4 ml./hr. with extreme diuresis, *e.g.* after injected magnesium sulphate. There are various degrees of oliguria and anuria; lobsters may be completely anuric for at least a month. Nephridial occlusion is not fatal to previously urinating lobsters over two- and three-week periods.

Contrary to possible supposition, the fluid volume of the lobster is not regulated by the volume containable within the exoskeleton. Fifteen to twenty-five ml. can always be injected into a normal animal (*c.* 0.5 kg.). The volume increase between emptiness and distension of the stomach (*c.* 10 ml.) and the nephridial bladders (*c.* 6 ml.) requires internal space into which these organs can expand. If one draws blood repeatedly, one sees directly a loss of blood volume which is not repaired in a few hours or even days to the initial volume. A lobster may be mobile and active, and yet from it blood can be drawn only slowly in contrast to the normal situation where blood can be drawn rapidly. Through the transparent ventral surface of the tail one can see the reduced blood volume.

In the normal animal there are about 24 ml., principally a sodium chloride solution, flowing through the animal and out as urine. This is at least several times the amount that is absorbed from the stomach. Indeed, urine at the above rate can be formed with lobsters with empty stomachs. It would appear that this sodium chloride solution must enter through the gills. Experiments with injected sodium chloride solutions (hypo- or hypertonic) with nephridial occlusion showed that water and sodium chloride can pass quickly (several hours) in either direction through the gills. Iodine also passes in either direction through the gills. Isotopic flux studies are necessary to define these parameters. The minimal net flux of water and sodium chloride is the urine flow, and this flow means that a sodium

chloride solution is moving inwardly across the gills faster than it is moving outwardly across the gills.

The inward direction of this flow of a sodium chloride solution seems to be governed by the blood proteins. Anuria and oliguria were associated with low blood protein as determined by the copper sulphate specific gravity method or by the weight per volume of blood. A specific gravity below 1.029 accompanied poor urine formation. So-called normal specific gravities were in the 1.032–1.033 range. In some anuric lobsters, erratic transitory flows of urine could be induced by the infusion of hypo- or hypertonic solutions (Na, Mg, Cl, SO_4). The transfusion, however, of 10–15 ml. of serum from urinating lobsters into anuric lobsters induced permanent urine flows, *i.e.,* the regular production of urine over a subsequent one week's test period (8 animals). Osmolar measurements by the Thermistor method (Table I) always gave isotonic or slightly hypertonic, never hypotonic, values for the blood as compared with sea water. The withdrawal of large amounts of blood from urinating lobsters, *e.g.* 0.1 of the blood volume (see Burger and Smythe, 1953), did not check urine formation, apparently because the blood did not become markedly diluted. With a 1200-gram lobster with a blood specific gravity of 1.032, the removal of 24 ml. of blood did not check urine formation nor was the specific gravity of the blood lowered 1.5 hours later. With a 650-gram animal, blood protein sp. gr. 1.0335, the specific gravity one and five days after the removal of 10 ml. of blood was 1.032.

Urine formation could not be correlated with hemocoelar blood pressure, a rise in which is accompanied by a rise in arterial pressure (Burger and Smythe, 1953). Hemoconcentration, resulting from keeping lobsters in the air, suppresses urine formation. In view of the above transfusion experiments, it would appear that the non-diffusible molecules of the blood draw in water principally through the gills, and this water is bailed out by the nephridium as urine. With this water moves sodium chloride. Ions to which the gills are not readily permeable, as well as some sodium chloride solution, enter from the stomach and leave through the nephridium. The nephridium keeps the blood volume below the fluid capacity of the shell. The constant nephridial removal of water from the blood should slightly raise the osmotic pressure of the blood. Since the blood circulates rapidly through the gills (Burger and Smythe, 1953), a very slight gradient should be enough to extract water from the external medium. The nephridium, however, is not merely an organ for maintaining fluid volume. It is capable of secreting some organic and inorganic substances and of restraining completely or partially other constituents of the blood. In short, it acts like a kidney. The all-over pattern seems to be one where substances are carried away by a high urine flow rather than by a powerful concentration of secreted substances. Only with phenol red, and only with very low blood concentrations was nephridial concentration marked. Since anuric lobsters live for at least a month without eating or drinking and since electrolytes such as magnesium do not build up in the blood, there is presumably no net inward flow of fluid as is found in the urinating animal. If the nitrogenous waste is ammonia, this can be lost through the gills. While there is no evidence that the lobster forms urea, exogenous urea is lost through the gills, and not through the nephridium. The absence of any volatile ammonia in the urine and the lack of any clear U/P difference in NPN make it dubious that the kidney is concerned with primary nitrogen excretion, although the occurrence of a persistent blood NPN indicates ni-

trogenous compounds which are not readily diffusible through the gills and which are removed in the urine. The gills, stomach, and nephridia form an interlocking system of entrance and exit, each with individual capacities.

This report gives no new information on the mechanism of urine formation. That the blood and urine are absolutely isosmotic (Table I) would be expected when one sees the extreme delicacy of the walls of the large nephridial bladders. Despite this delicacy, substances such as phenol red can be held at higher than blood concentrations. Pressure from the nephridial fluid must distend these bladders and one can imagine a small isosmotic filtration from bladder to blood, or the reverse when the blood pressure is raised.

From the work of Peters (1935) it is assumed that urine is formed by filtration. The ready passage of fish blood proteins and of inulin supports the filtration idea. Histologically, the nephridium seems more comparable to the aglomerular fish kidney where filtration does not occur. The nephridial arteries lie behind a wall of glandular epithelium and there seems to be no glomerulus-like structure or arrangement. This problem is worthy of further study.

Summary

1. A method is given for safely securing repeated evacuations of the nephridial bladders of the lobster, *Homarus,* thus permitting experimental analysis of nephridial function.

2. Routine chemical analyses for blood, urine, and sea water are given.

3. Experimental analysis shows that the nephridium can concentrate in the urine phenol red, para-aminohippurate, magnesium, and sulphate. At normal blood levels, it completely excludes glucose and Fiske-SubbaRow phosphate. Phlorizin blocks glucose retention. With high blood levels, the secretory or exclusion powers of the nephridium are swamped. Calcium is partially excluded from the urine. The nephridium is indifferent to inulin, bromsulfalein, dogfish hemoglobin and plasma protein, sodium and chloride. The ability of the digestive gland to concentrate phenol red and bromsulfalein in the digestive juice is noted.

4. Exogenous urea is lost through the gills.

5. The gills and carapace are relatively impermeable to magnesium and sulphate, and to phenol red, but are freely permeable to water, sodium chloride, and to exogenous urea.

6. In full-strength sea water, the distribution of sodium chloride between sea water, blood, and urine seems to be passive. In dilute sea water, experiments indicate that blood chloride is elevated in some active fashion, presumably by the gills. The nephridium, however, does not aid in the conservation of chloride. Chloride is lost at the elevated blood level. The nephridium does not seem to have powers to deal actively with sodium chloride.

7. The normal urine flow is approximately 1 ml./hr./0.5 kg., with wide variations. Water and sodium chloride for this flow enter largely through the gills although there may be intermittent contributions from the stomach. Magnesium and sulphate enter largely through the stomach. Calcium, in addition to that of sea water, is in the food. The lobster intermittently drinks sea water with food or on an empty stomach. The stomach contents are absorbed completely but not rapidly enough to furnish the fluid for the bulk of the urine flow. The daily

urinary excretion of magnesium roughly equals the magnesium found in 5 ml. of sea water. All test substances entered the blood from the stomach. In any study of sea water and blood, the gastric contribution to the chemistry of the blood, even for electrolytes, must be considered.

8. Lobsters from commercial pounds are frequently oliguric or completely anuric. Inability to form urine is not lethal, at least over a one-month period. Erratic transitory urine flows can be induced in anuric lobsters by the injection of various saline solutions. Normal urine flow can be induced in anuric lobsters by the transfusion of serum from one lobster to another. Apparently, substances such as blood proteins which are not diffusible through the gills draw in water which is bailed out by the nephridia. Osmolar measurements found the blood isotonic or slightly hypertonic, never ~~isotonic~~ hypotonic, to sea water.

9. While the nephridium has a range of capacities for dealing with individual substances (secretion, exclusion, partial exclusion, and a lack of partition power), its secretory capacities are not great and are masked easily by elevated blood levels. Nephridial removal of substances from the blood depends more on a flush-out principle, using large urine flows, than upon secretory powers. Some substances such as exogenous urea are lost by the gills and not by the nephridium. Together, the nephridia and the gills form an excretory system, each with individual capacities.

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